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A resorufin-based colorimetric and fluorescent probe for live-cell monitoring of hydrazine

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ABSTRACT

We report a novel colorimetric and red-emitting fluorescent probe for hydrazine detection based on resorufin platform. This OFF–ON fluorescent probe shows a large (117 nm) red-shifted absorption spectrum and the color changes from colorless to red upon addition of hydrazine in the aqueous solution, which can serve as a "naked-eye" probe for hydrazine. Moreover, this probe also shows a significant fluorescence increase (~16 folds) and excellent linear relationship at physiological pH. Utilizing this sensitive and selective probe, we have successfully detected hydrazine in living cells.

1. Introduction

Hydrazine (NH_2NH_2) is well known as various rocket fuels and low-power monopropellant for its flammable and detonable characteristics (Zelnick et al., 2003). Hydrazine, which as a highly reactive base and strong reducing agent, is also a very useful building block in organic synthesis of pesticides, pharmaceuticals, photography developers, and textile dyes in various chemical industries (Yamada et al., 2003). However, hydrazine is highly toxic and dangerously unstable, can easily lead to serious environmental contamination during the process of manufacture, transport, usage and disposal. Moreover, hydrazine can also cause serious damage to the liver, lungs, kidneys, the nervous system and other human health problems when it has been absorbed by oral, dermal, or inhalation routes of exposure (Bollard et al., 2005). Therefore, the development of reliable, sensitive, selective and convenient analytical methods for hydrazine is very important and highly desirable.

Several conventional analytical techniques have been developed for hydrazine detection, such as chromatography and electrochemical detection (Elder et al., 2011; Batchelor-McAuley et al., 2006; Umar et al., 2008). However, these methods required special equipment, complicated sample preparation processes, destruction of tissues or cells, which were unsuitable for the analysis of hydrazine in living systems. Recently, much attention has been paid to develop fluorescent probes for detecting hydrazine (Ensafi and Rezaei, 1998). To the best of our knowledge, several fluorescent probes for hydrazine have been reported (Chen et al., 2008; Choi et al., 2011; Fan et al., 2012; Choi et al., 2013; Hu et al., 2013; Goswami et al., 2013; Lee et al., 2013). In these probes, some could be used for imaging of hydrazine in live cells, while others were only applicable for *in vitro* detection. Moreover, most of these current probes could only be utilized in much higher organic cosolvent (> 40%, v/v) or low pH (pH < 5) condition, which would limit their application in physiological condition. So far, chemical small-molecular fluorescent probes for hydrazine are still very limited.

Among the many sophisticated signaling systems, there are many fluorescent chemical probes reported for the chemodosimetric detection of various analytes by selective chemical transformation and cumulative signaling effects (Jun et al., 2011). Mainly, the deprotection of a specific protecting group has been utilized to develop a series of fluorescent dyes, such as the silyl ether for fluoride (Bhalla et al., 2010), the boronate for hydrogen peroxide (Dickinson and Chang, 2008), and the thioacetals for mercury ion (Cheng et al., 2010). Goswami et al. recently reported a bromo-ester derivative of 2-(2-Hydroxyphenyl) benzothiazole (HBT) can be utilized as a chemogenic probe for hydrazine by the deprotection process of subsequent substitution–cyclization–elimination sequence based on ESIPT mechanism (Goswami et al., 2013). However, the short-wavelength excitation (λ_{ex}=300 nm) and the poor biocompatible (CH_3CN/H_2O, 2/3, v/v) of this probe would limit its biological applications in the complicated living systems as the strong absorption and autofluorescence of biomolecules background would lead to low signal-to-noise ratios if the

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excitation of the probe at the short-wavelength region (Yuan et al., 2012). Moreover, the short-wavelength light could lead to severe photodamage to cell or tissue samples and reduced tissue penetration (Yuan et al., 2013). Resorufin as a signaling fluorophore group was an ideal mode for constructing OFF–ON fluorescent and chromophoric chemosensors because of its excellent photophysical and photochemical properties, such as pronounced colorimetric possibilities, good water-solubility and easy fluorescence quenching *via* 7-hydroxy substitution (Gao et al., 2003; Kim and Hong, 2007; Choi et al., 2010a, 2010b; Choi et al., 2010a, 2010b). Actually, some fluorescent probes have been successfully developed based on resorufin optical signaling system to track important chemical species. Typical examples are Chang et al. developed a new resorufin-based probe for H_2O_2 *via* debornation (Miller et al., 2007) and Ma et al. described a benzoyl peroxide probe also based on resorufin fluorophore (Chen et al., 2012). We envision that a novel bromo-ester of resorufin can be developed as a hydrazine reporter through the selective deprotection process, which can react with hydrazine to release the free resorufin to generate the significant change of fluorescence. Based on above consideration, using this transformation, we herein reported a resorufin-based colorimetric (from colorless to red) and red-emitting hydrazine fluorescent probe (HFP) with 117 nm absorption red-shifted for monitoring of hydrazine in aqueous solution and live-cell at physiological pH.

2. Experimental

2.1. Materials and instrumentations

All chemicals were purchased from commercial suppliers and used without further purification. Chinese hamster ovary (CHO) cells were purchased from the Committee on type Culture Collection of Chinese Academy of Sciences. Chromatographic purification of products was accomplished by using forced-flow chromatography on silica gel (300–400 mesh). Thin layer chromatography was performed on EM Science silica gel 60 F254 plates (250 μm). Visualization of the developed chromatogram was accomplished by UV lamp. Nuclear magnetic resonance (NMR) spectra were acquired on Bruker DRX-400 operating at 100 MHz for ^{1}H NMR and ^{13}C NMR; residual protio solvent signals were used as internal standards for calibration purposes. Data for ^{1}H NMR are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), integration, coupling constant (Hz). TOF EI Mass was performed by Mass Spectrometry Facility at Nanjing University. All fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence Spectrophotometer and Hitachi Fluorescence Spectrophotometer F-7000. All imaging experiments were performed on a fixed cell DSU spinning confocal microscope (Olympus). The pH measurements were carried out on a HI 2221 calibration check pH/ORP meter.

2.2. General procedure for analysis

Stock solution of hydrazine and other analytes were prepared in ultra-pure H_2O, stock solution of HFP (1 mM) were prepared in CH_3CN, which was diluted to the required concentration for measurement. All fluorescence measurements were carried out at room temperature on a Hitachi Fluorescence Spectrophotometer F-7000. The samples were excited at 560 nm with the excitation and emission slit widths set at 5.0 nm. The emission spectrum was scanned from 570 nm to 660 nm at 1200 nm/min. The photomultiplier voltage was set at 400 V.

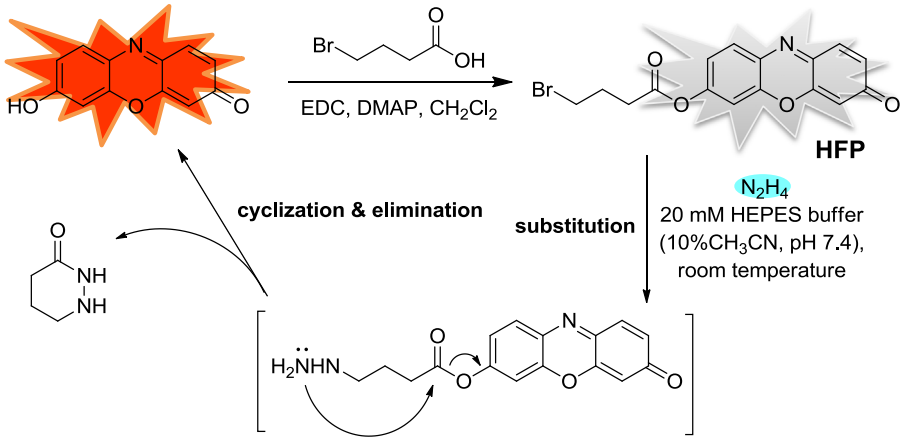
2.3. Cell culture and fluorescence imaging

Chinese hamster ovary (CHO) cells were grown up in DMEM medium with 10% fetal bovine serum/penicillin/streptomycin in a 5% CO_2 atmosphere at 37 °C. Cells were then seeded on Coverglass-Bottom confocaldish and continuously incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. After CHO cells were incubated with HFP (10 μM) for 20 min at 37 °C, media was removed and cells were washed with PBS buffer three times again to remove any probe left in solution to optimize the background signal. Then, cells were added in fresh media and incubated with hydrazine (0 μM and 20 μM) for another 60 min at 37 °C before imaging. All imaging experiments were performed on a fixed cell DSU spinning confocal microscope (Olympus). Excitation and emission monitored used Alexa Fluor 568 filters. Imaging performed using X40 objective, and captured using Slidebook software. For all experiments, solution of HFP was prepared in DMSO (1 mM) and diluted into DMEM to the desired working concentration (10 μM, 1% DMSO). Hydrazine was diluted into DMEM to the desired working concentrations (20 μM) from a 1 mM stock solution.

2.4. Synthesis of HFP

2.4.1. 3-Oxo-3 H-phenoxazin-7-yl-4-bromobutanoate

To a solution of Resorufin (426 mg, 2 mmol) and 4-Bromobutyric acid (367 mg, 2.2 mmol, 1.1 equiv.) in dichloromethane (5 ml) was added N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC · HCl, 1.75 equiv.) and 4-Dimethylaminopyridine (DMAP, 0.1 equiv.). The reaction mixture was allowed to stir at room temperature for 15 h. Then the solvent was evaporated under reduced pressure and the obtained residue was purified



Scheme 1. Synthesis of HFP and the proposed mechanism of the response of HFP to hydrazine.